行政院國家科學委員會
「提升私大研發能量」專案計畫
第一期期中成果發表會

學習記憶障礙
之中醫藥治療研究

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王慧如、李珮端

學習神經科學研究中心

分子生物基因研究室〈子計畫二〉

老化基因測定

篩選中藥益智成分

動物學習評估

學習行為研究室〈子計畫一〉

- 活體動物學習研究組
- 離體神經細胞電生理傳導研究組
- 臨床試驗組

製劑暨藥物動力學研究室〈子計畫三〉

神經電生理傳導測定

智能療效評估

中藥益智藥開發

總計畫及子計畫名稱

計畫項目	計 畫 名 稱	主持人
總計畫	學習記憶障礙之中醫藥治療研究	謝明村
子計畫一	中藥地黃成分catalpol對雌雄鼠暨停經雌鼠學習記憶障礙及腦部中膈海馬神經迴路角色之評估	謝明村
子計畫二	中藥對scopolamine或Aβ1-42誘發神經變性時特殊基因蛋白及細胞活性之影響	許朝添
子計畫三	改善學習記憶障礙中藥之藥動學、藥劑學研究及其對大鼠海馬迴Ach釋放之影響	李珮端

癡呆、健忘及失憶

	痴呆 （Dementia）	健忘 （Amnesia）	失憶 （Forgotten）
定義	又稱『失智』 腦機能逐漸退化之疾病	對事物遺忘，無法再記取	對事物忘記，但可 經由訓練而記得
分類	退化性痴呆（阿爾滋海默氏症） 腦血管性痴呆（多發性中風） 假性癡呆（憂鬱症）	進行性健忘（痴呆初期） （Anterograde amnesia） 退行性健忘（痴呆末期） （Retrograde amnesia）	無
治療	退化性痴呆：tacrine 腦血管性痴呆：propentofylline	如前	智能訓練， 不需治療

學習行為研究成果

- 大鼠痴呆模式之建立
- 大鼠學習能力之測定
- 大鼠腦內單胺濃度之測定
- 大鼠海馬神經細胞電位興奮之測定

動物痴呆模式之建立

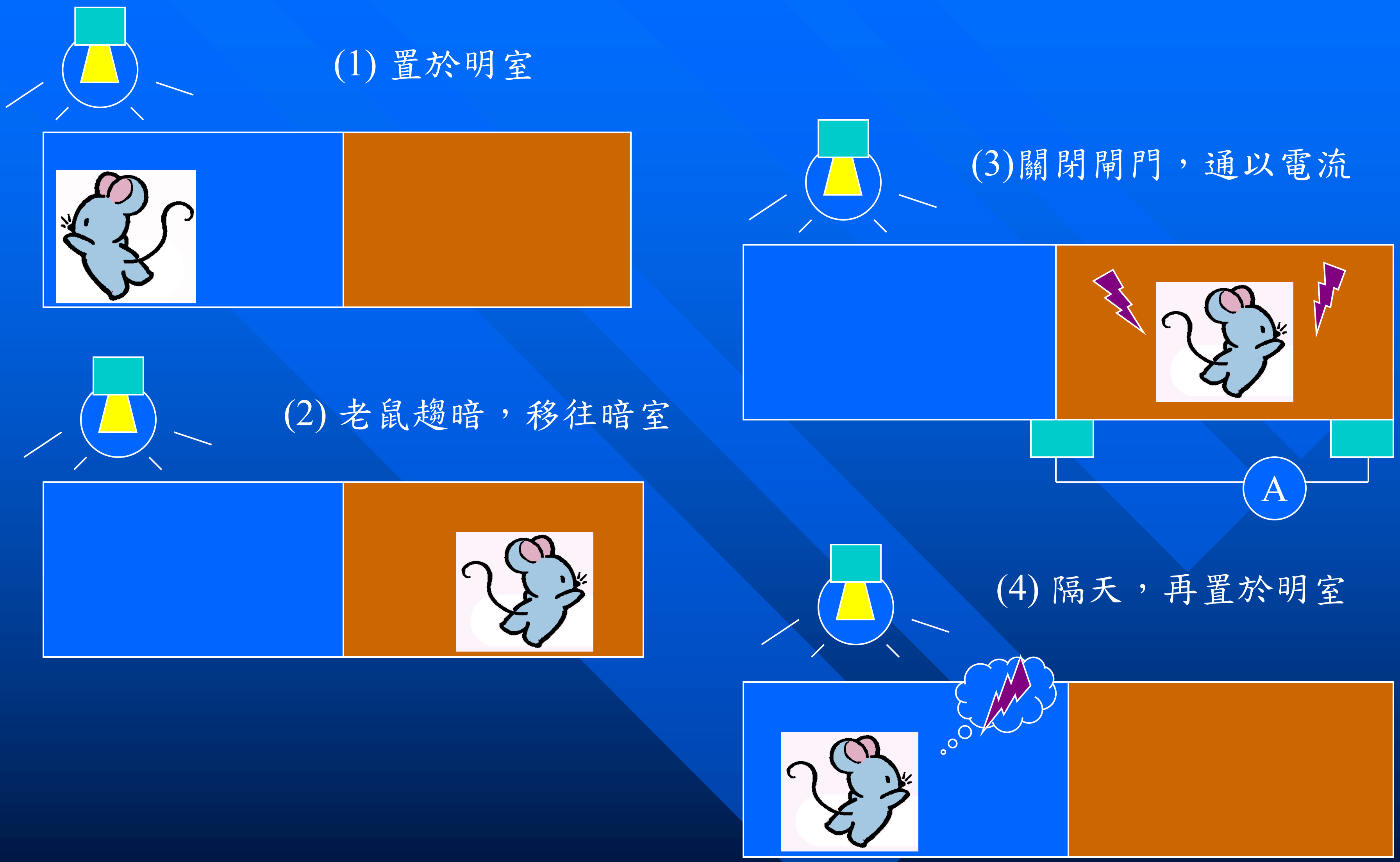
- 腦血管性痴呆模式
  - 雙側頸動脈結紮誘發腦缺血模式

    （Caldwell et al., Brain Res. 1997）
- 退化性痴呆模式：
  - β Amyloid peptide 1-40模式

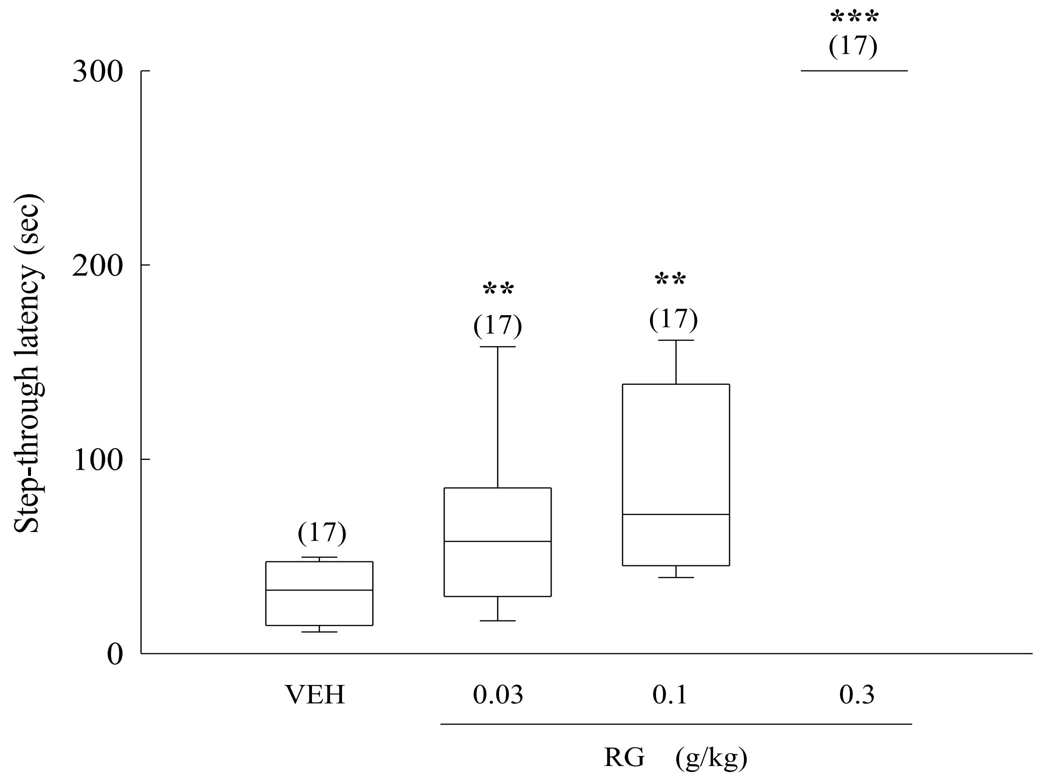
    （Tanaka et al., Eur J Pharmacol 1998）

動物學習模式之分類

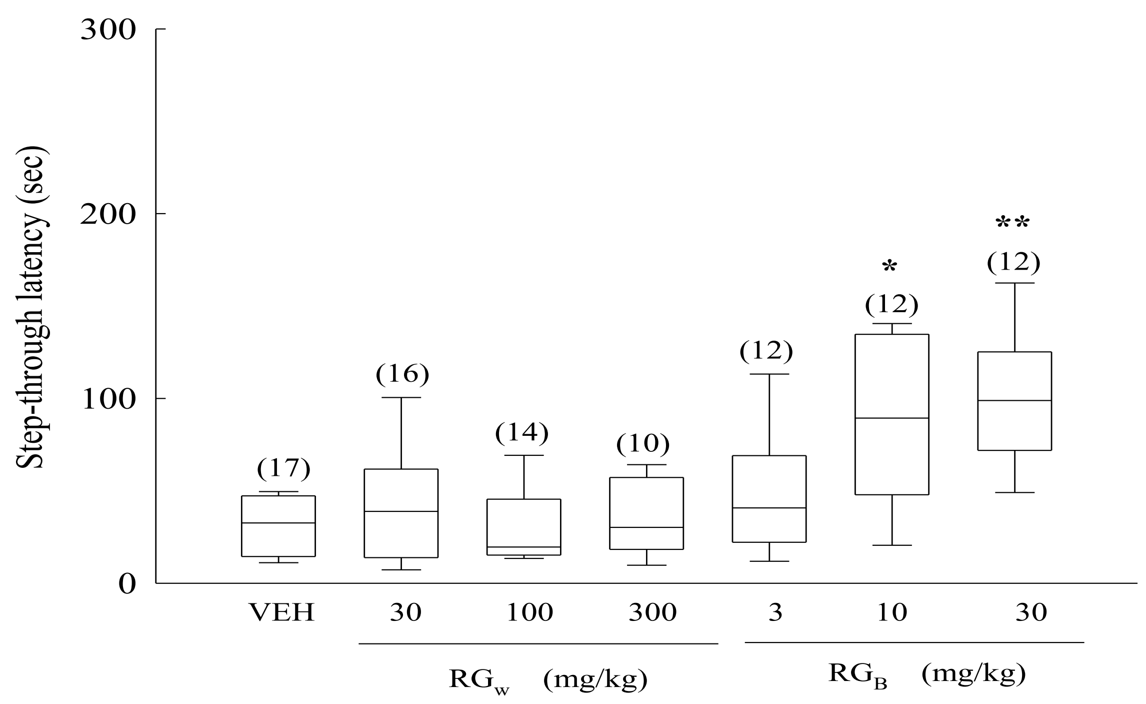
學習步驟分類	刺激原型態	動物行為	動物模式	機制
正向性強化 （Positive reinforcement）	報償 食慾性刺激	獲得食物	八臂式迷宮 （8-radial arm maze）	週邊暨中樞 hypothalamus 及 mesolimbic DA pathway 神經系統
負向性強化 （Negative reinforcement）	害怕 厭惡性刺激	迴避刺激	主動迴避反應 （Active avoidance） 水迷宮（water maze）	週邊暨中樞 hippocampus 及 amygdala 神經系統
懲罰 （Punishment）	害怕 厭惡性刺激	抑制本能	被動迴避反應 （Passive avoidance）	


(1) 置於明室
(2) 老鼠趨暗，移往暗室
(3)關閉閘門，通以電流
A
(4) 隔天，再置於明室

地黃甲醇粗物對正常大鼠被動迴避學習反應之作用



地黃甲醇粗物分層萃取物對正常大鼠被動迴避學習反應之作用



地黃成分 catalpol 之分離流程

地黃
Rehmannia glutinosa

↓ 甲醇抽取

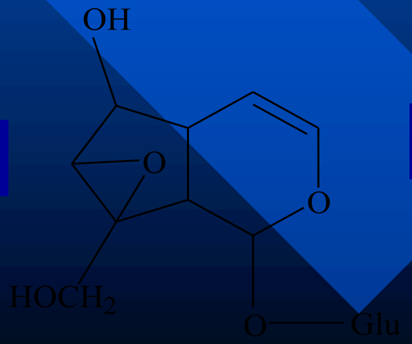
地黃甲醇粗抽物

水溶解再以正丁醇萃取

地黃正丁醇層萃取物 | 地黃水層萃取物

層析分離

Catalpol



梓醇

地黃成分 catalpol 對健忘及痴呆動物水迷宮空間操作之影響

水迷宮訓練流程

- 空間操作〈Spatial performance〉
  - 安全平台低於水面 1 cm
  - 1～3 天，每天訓練4次，每次120秒
- 參考記憶〈Reference memory〉
  - 第3天最後一次訓練後4小時， 安全平台移除
  - 訓練1次60秒
- 工作記憶〈Working memory〉
  - 安全平台移至對角象限，低於水面 1 cm

Water Maze Version 3.20

Information:

<Esc> Return to Main Menu
<F1> HELP (toggle)
<F2> Toggle Analysis Results
<F3> Print all trial Maps from a loaded experiment
<F4> Make analyzed data files
<F5> Artifact Filter: [OFF]

Hit <RETURN> on an empty path to display experiments (*.exp)

Select experiment,then hit the <RETURN> key to show trials.

Select trial,<RETURN> to load

RELOADING RAW DATA

PATH\EXPERIMENT= d:\lez\1207\1207.EXP

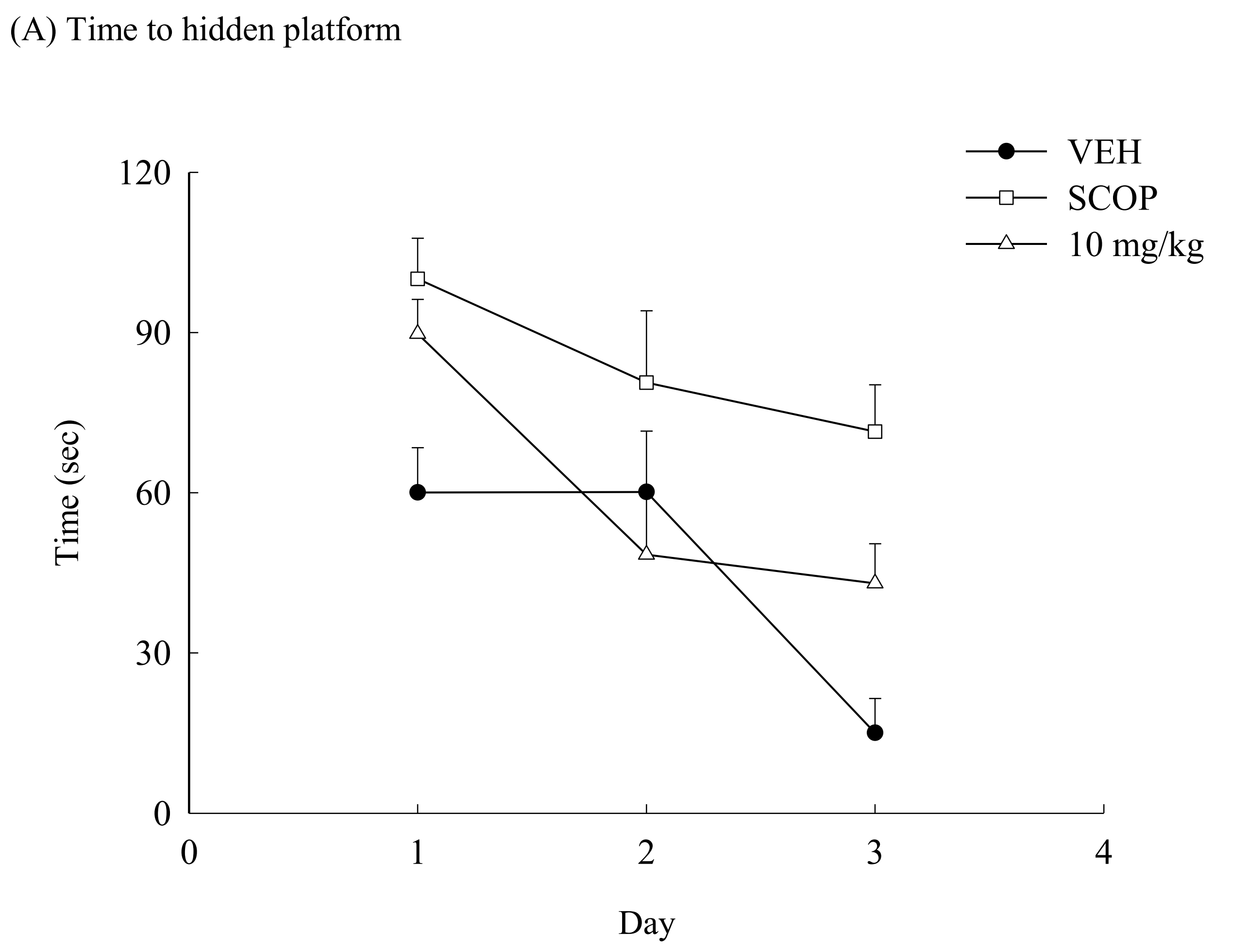
Listing Trials: Trial= cat0111 File= 120716.RAW

00. veh11	01. veh12	02. veh13	03. veh14	04. veh21
05. veh22	06. veh23	07. veh24	08. veh31	09. veh32
10. veh33	11. veh34	12. veh41	13. veh42	14. veh43
15. veh44	16.[cat0111]	17. cat0112	18. cat0113	19. cat0114
20. cat0121	21. cat0122	22. cat0123	23. cat0124	24. cat0131

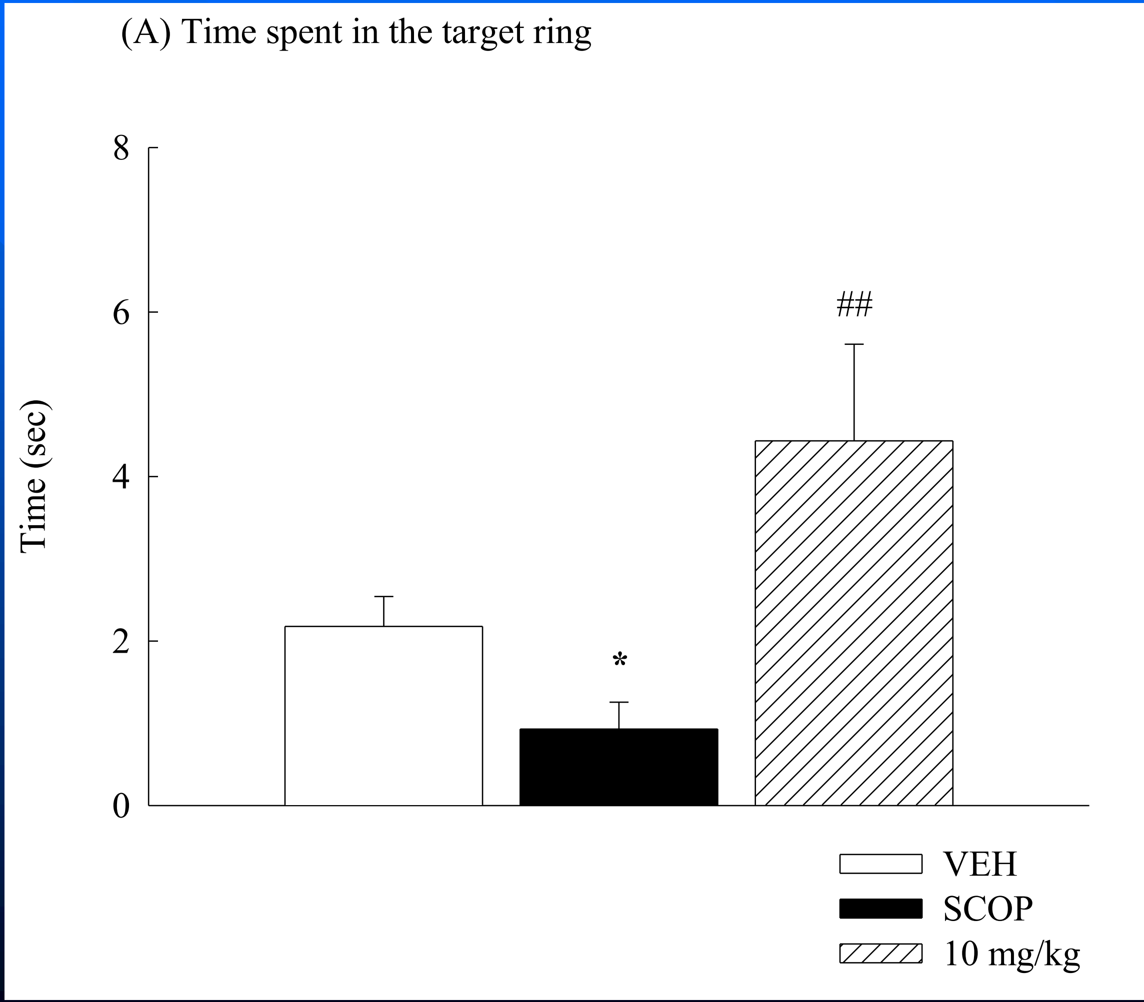
水迷宮記憶型態及相關腦部位

操作型態	記憶型態	相關腦部位
空間操作	學習獲得	Hippocampus Amygdala
參考記憶	記憶再現	Hippocampus Cortex
工作記憶	再學習	Hippocampus
	記憶再現	Hippocampus Cortex

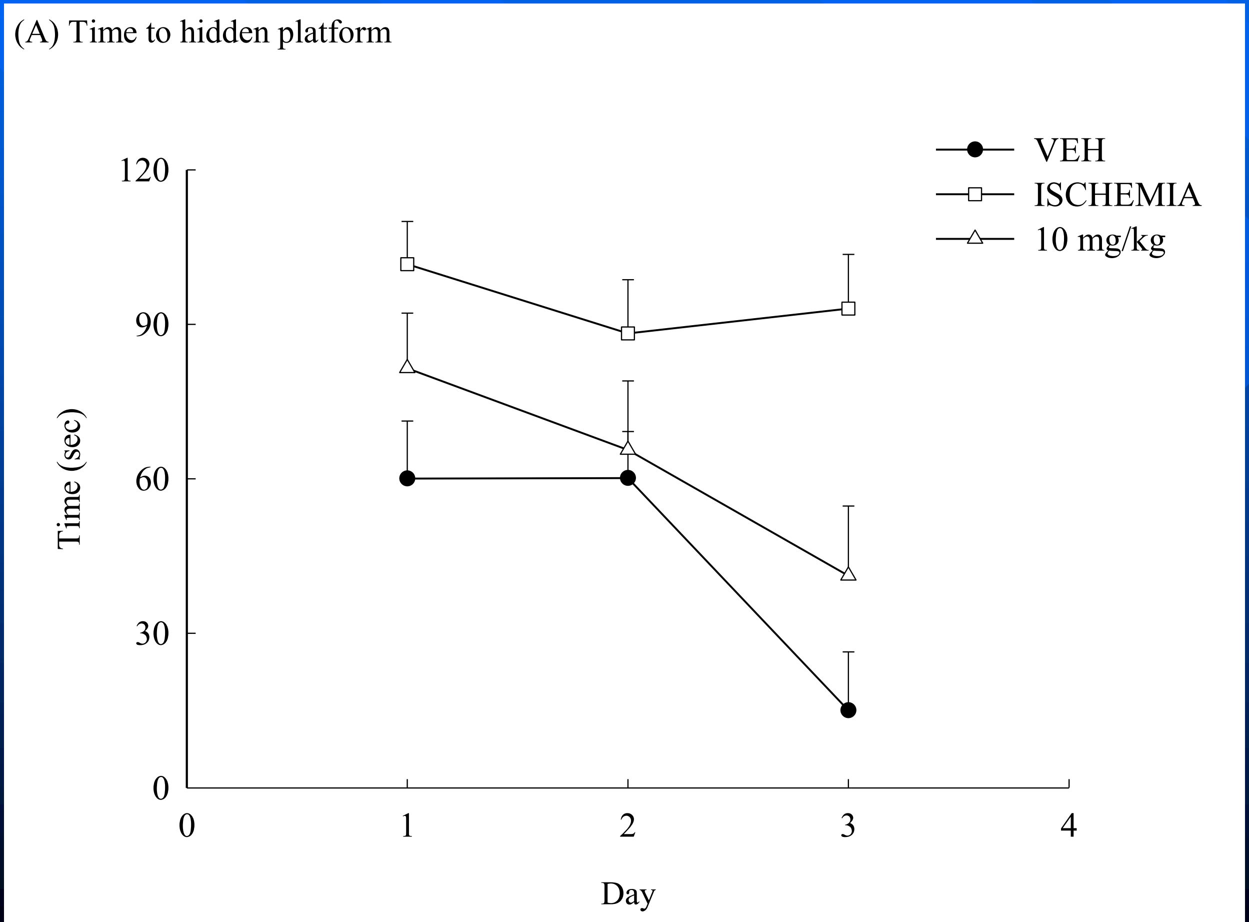
地黃成分catalpol對scopolamine誘發大鼠水迷宮空間操作障礙之作用



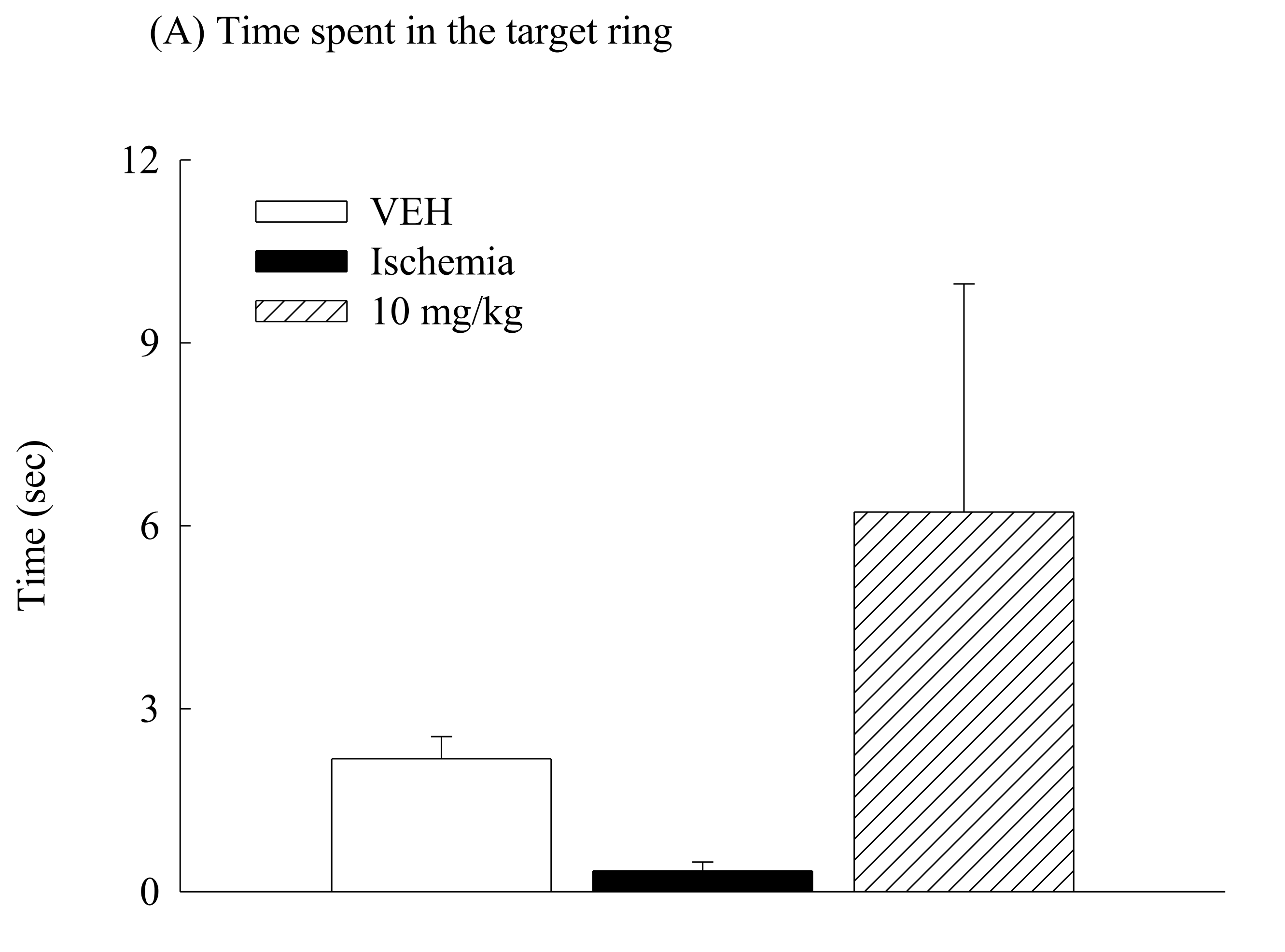
地黃成分catalpol對scopolamine誘發大鼠參考記憶障礙之作用



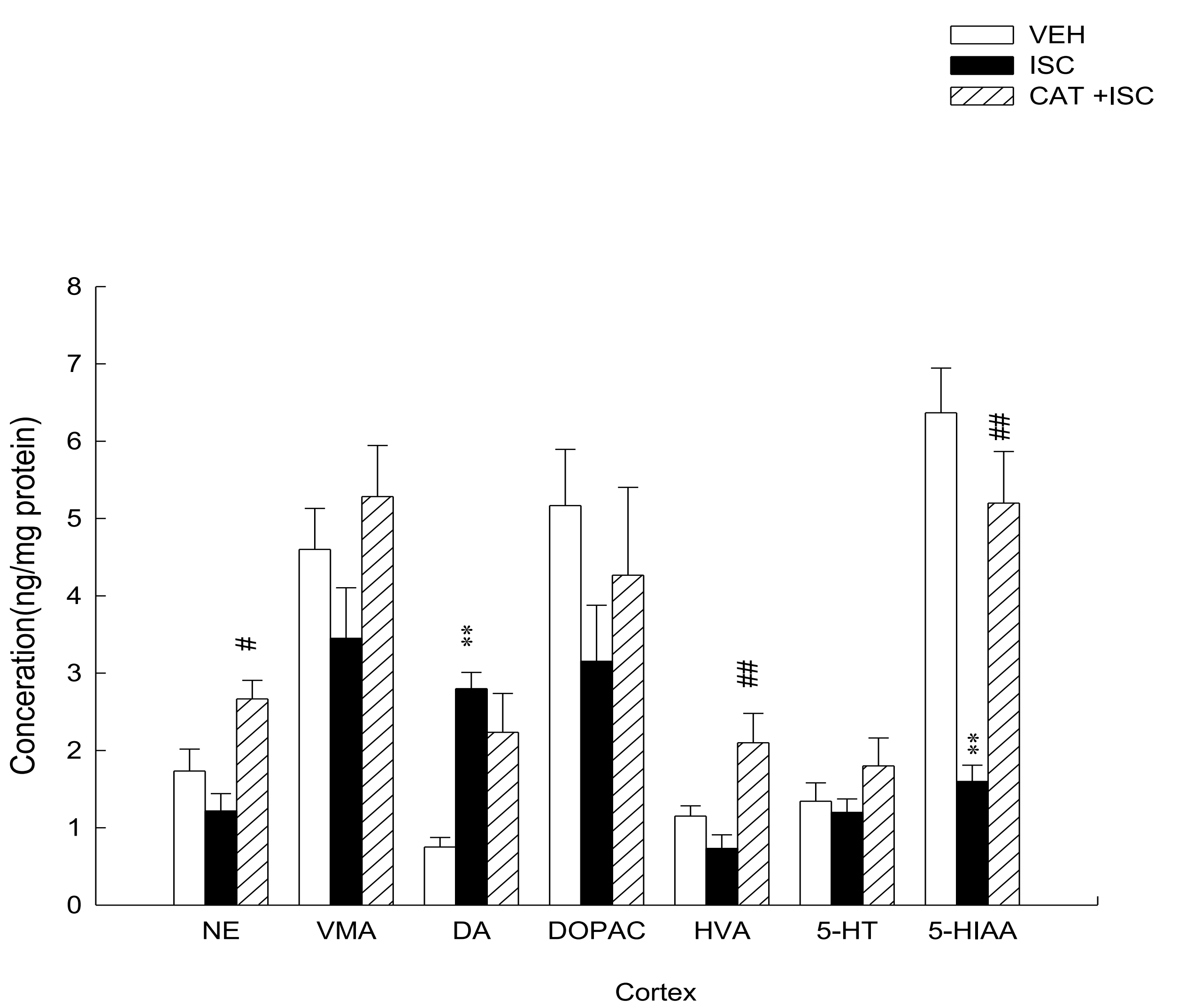
地黃成分catalpol對腦缺血大鼠水迷宮空間操作障礙之作用



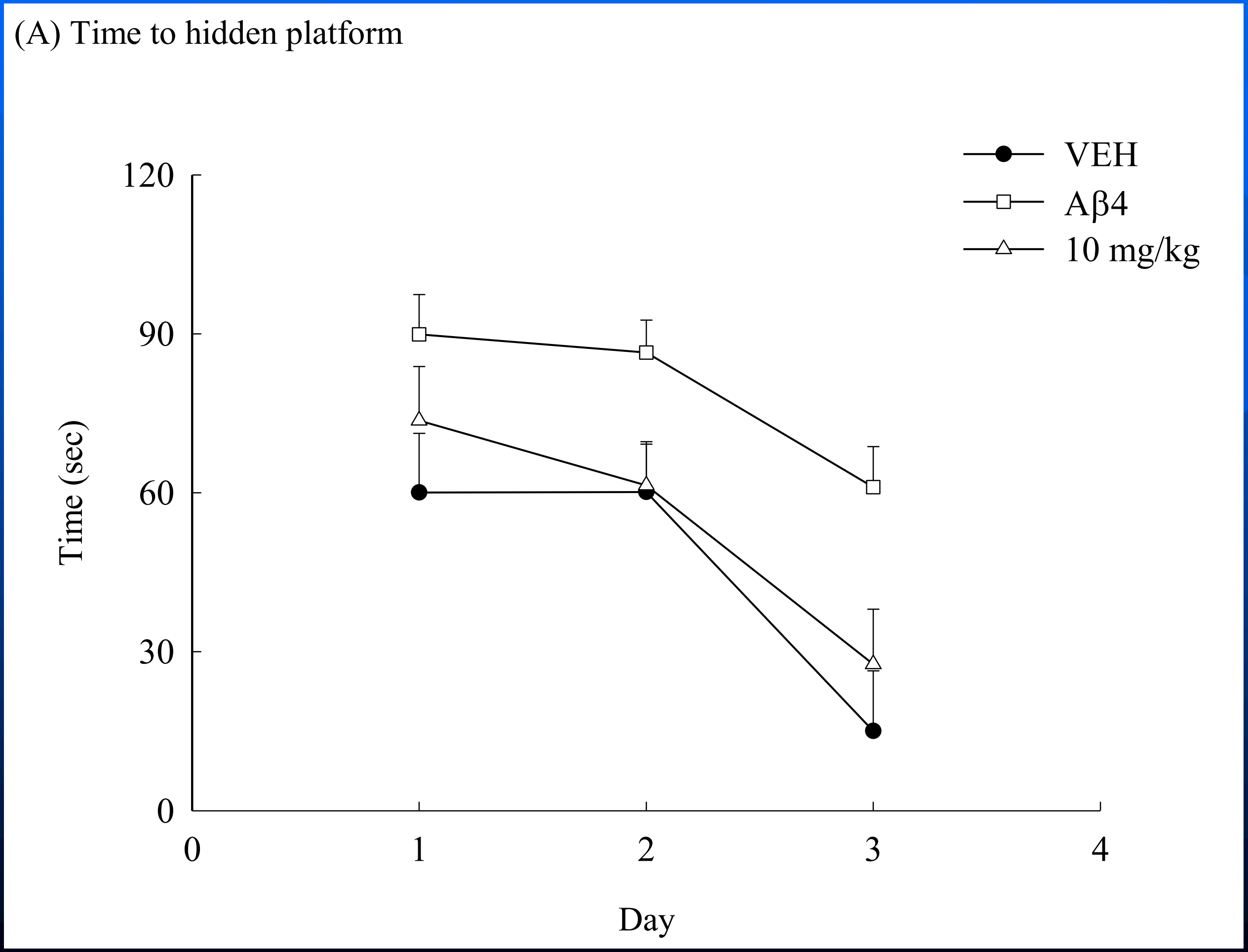
地黃成分catalpol對腦缺血大鼠水迷宮參考記憶障礙之作用



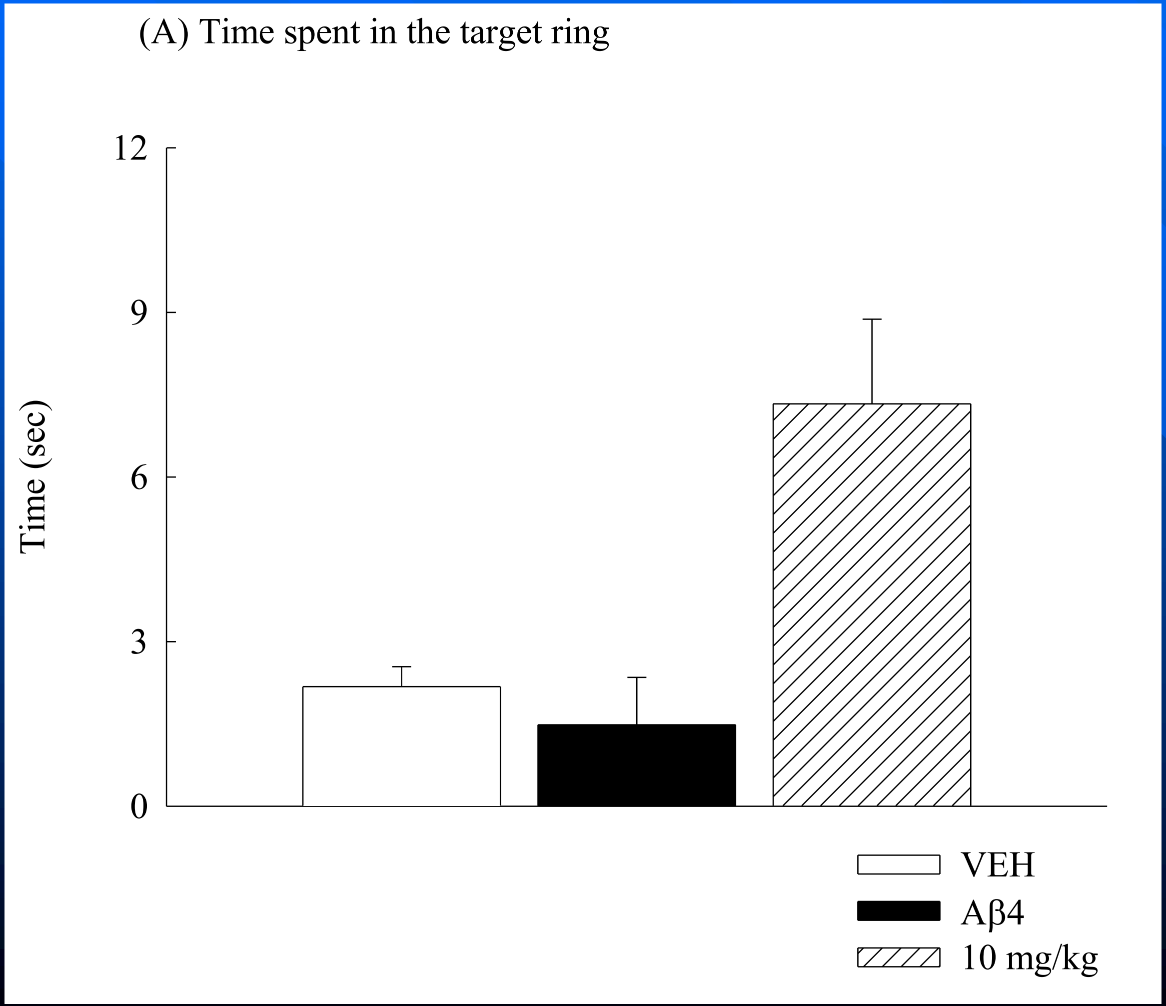
地黃成分catalpol對腦缺血大鼠導致大鼠皮質區單胺濃度變化之影響



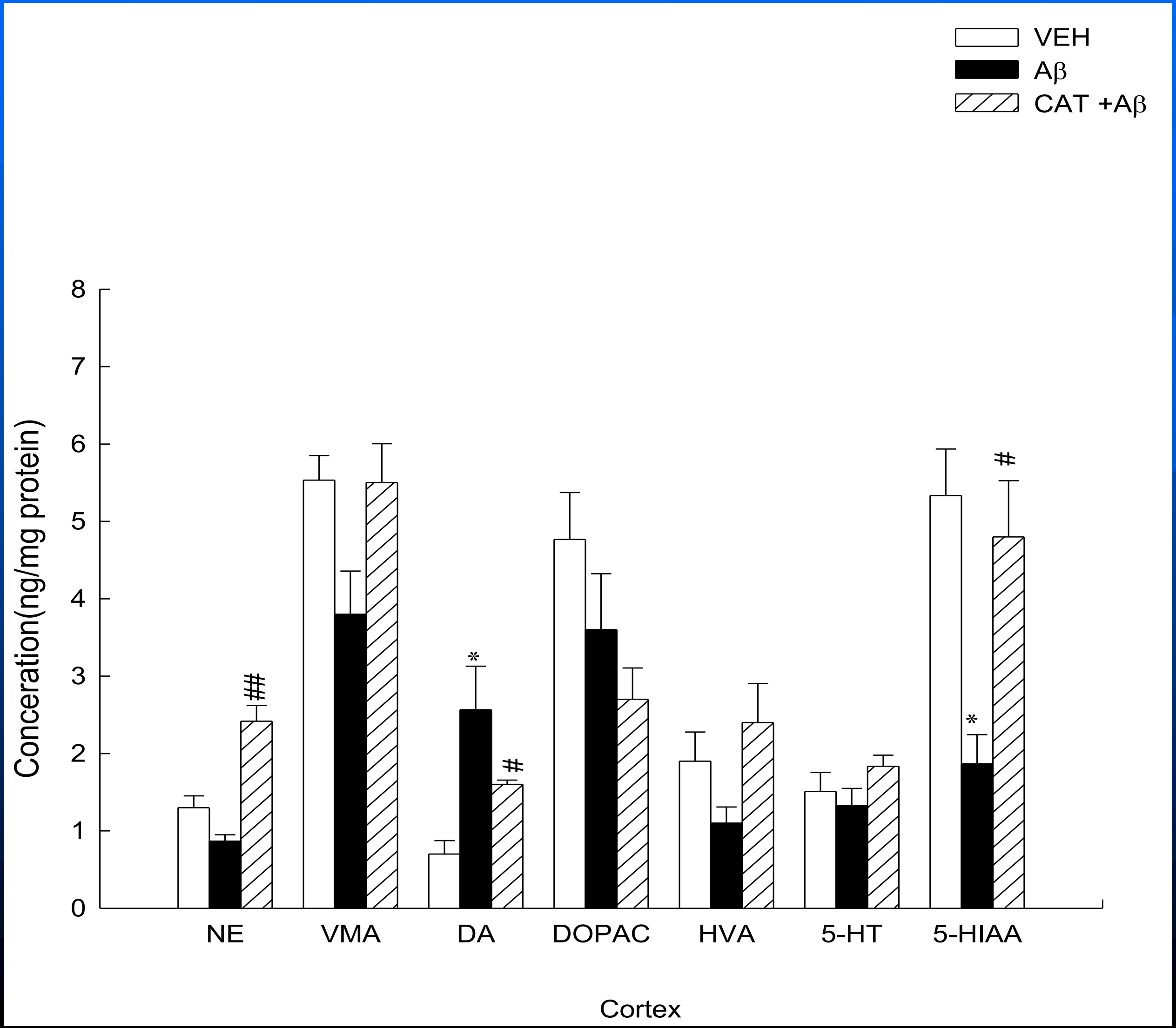
地黃成分catalpol對 Aβ 4 誘發大鼠水迷宮空間操作障礙之作用



地黃成分catalpol對 Aβ 4 誘發大鼠參考記憶障礙之作用



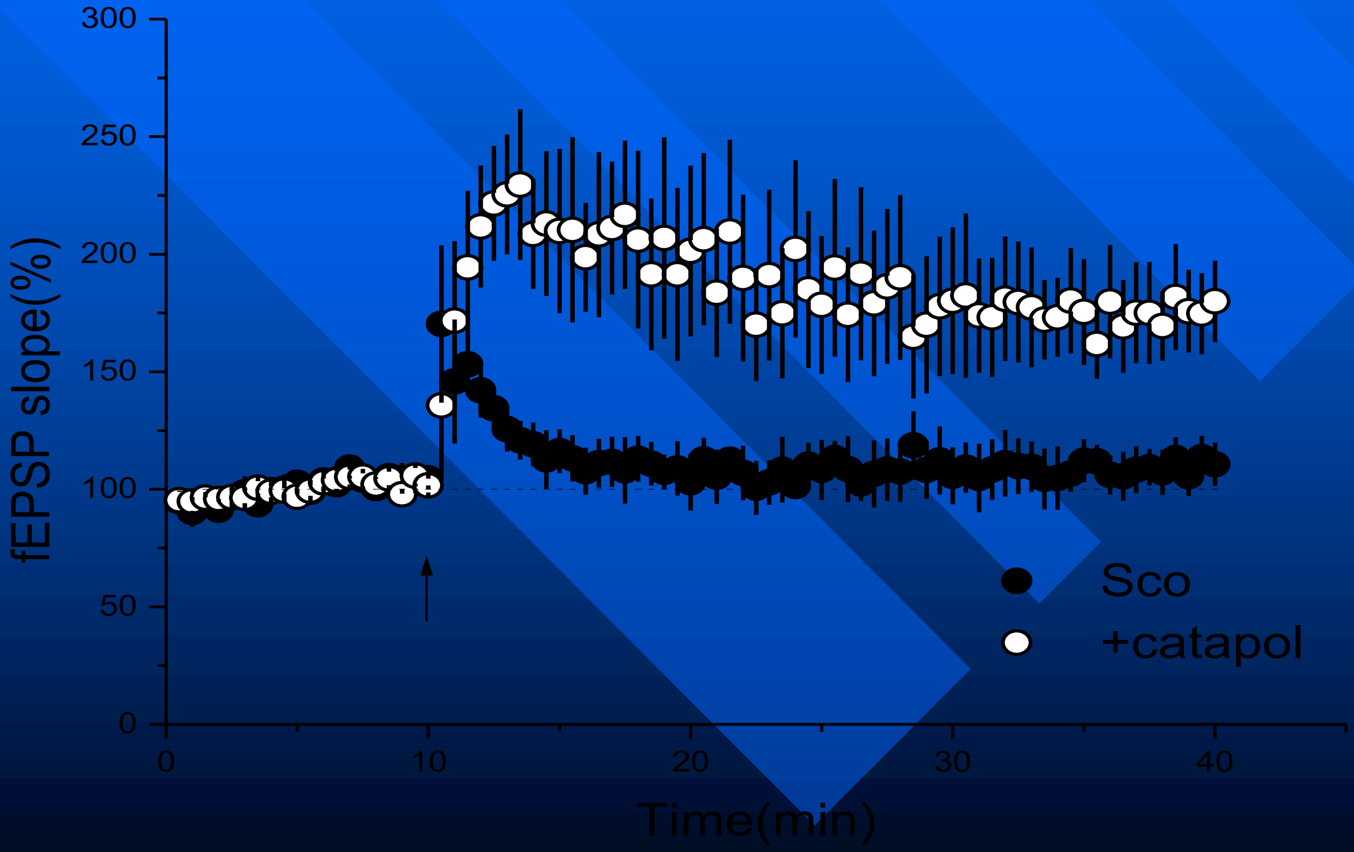
地黃成分catalpol對 Aβ 4 導致大鼠皮質區單胺濃度變化之影響



地黃成分catalpol對痴呆大鼠腦部單胺濃度變化之影響

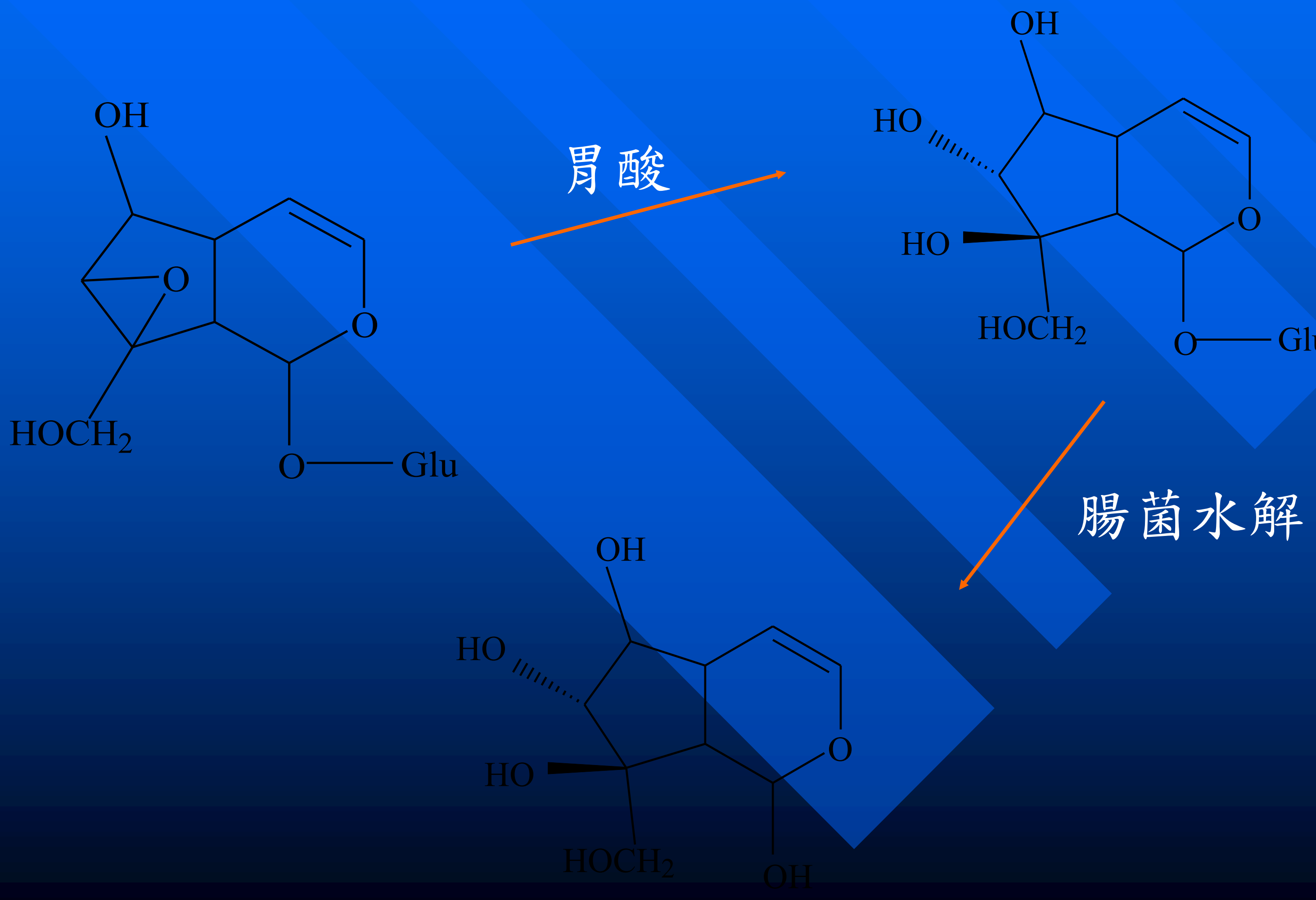
		Cortex			Hippocampus		
		NE	DA	5-HT	NE	DA	5-HT
Ischemia	Vehicle	⇩	⇧⇧	—	⇩	⇧	—
	Catalpol	↑	—	—	↑	—	—
Aβ 4	Vehicle	⇩	⇧	—	⇩	⇧	—
	Catalpol	↑	↓	—	↑	↓	—

地黃成分catalpol對大鼠海馬神經細胞電位興奮之影響

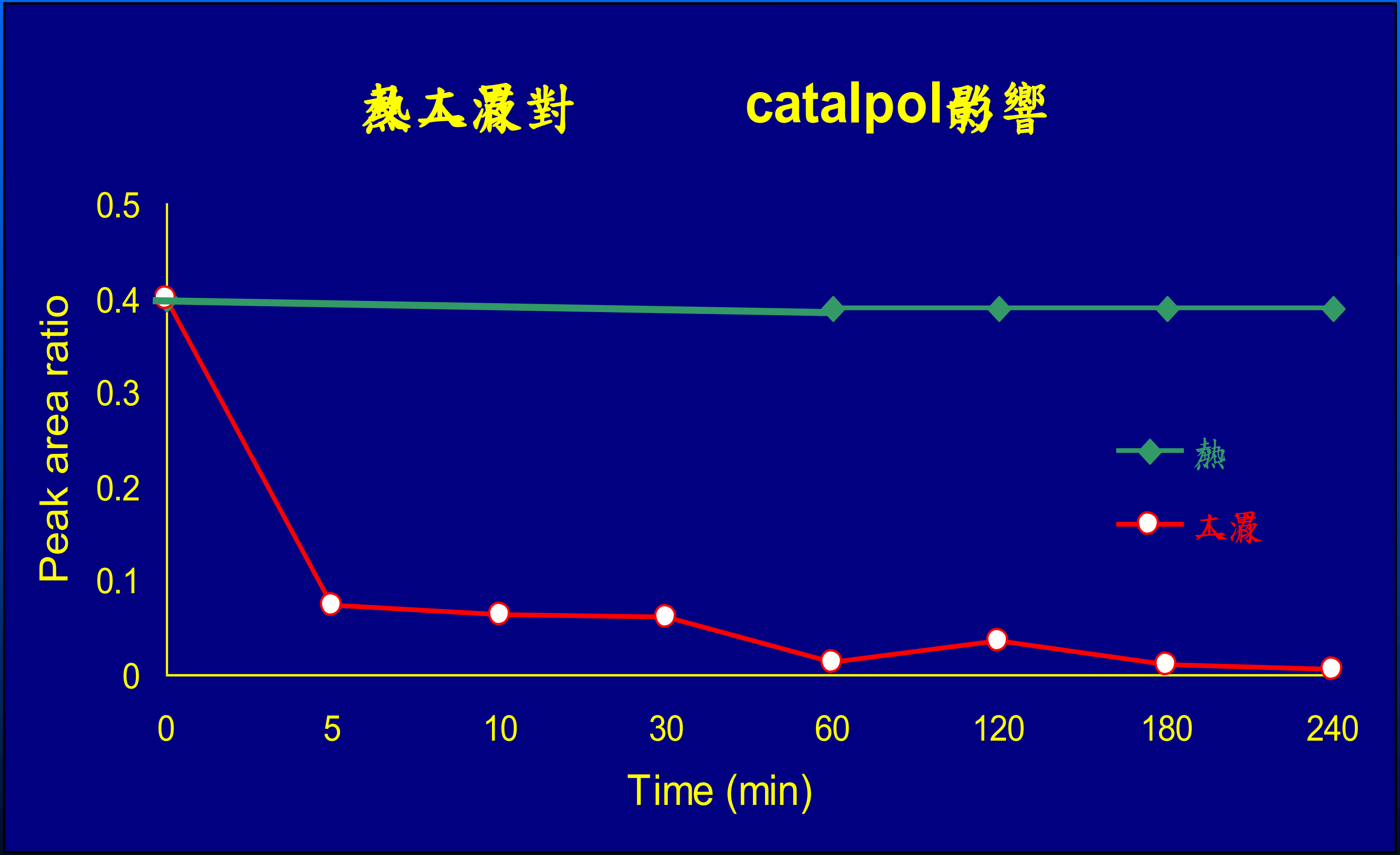


藥物動力學研究成果

Catalpol 之代謝

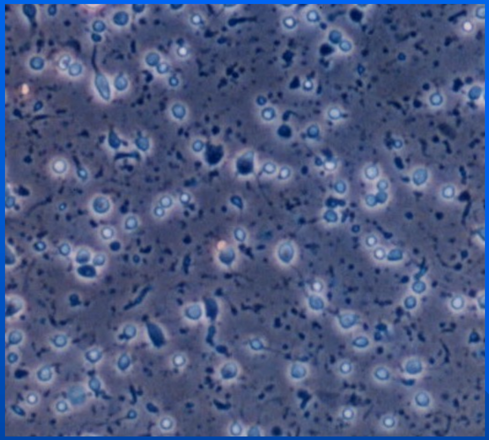


熱及人工胃液對 catalpol 的影響

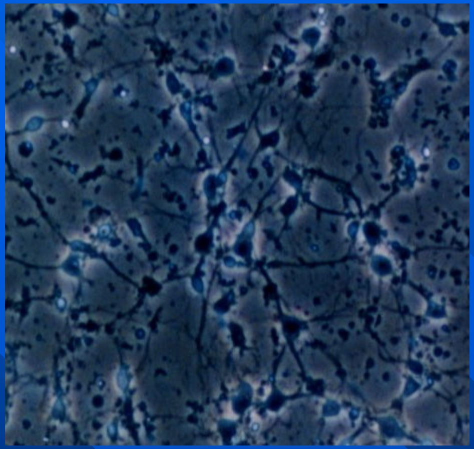


分子生物基因研究成果

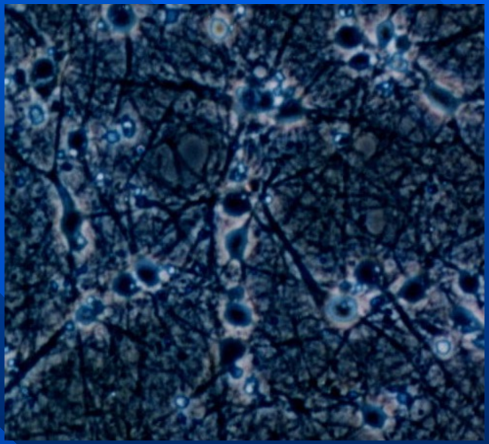
海馬迴神經細胞初代培養模式



培養 1 天

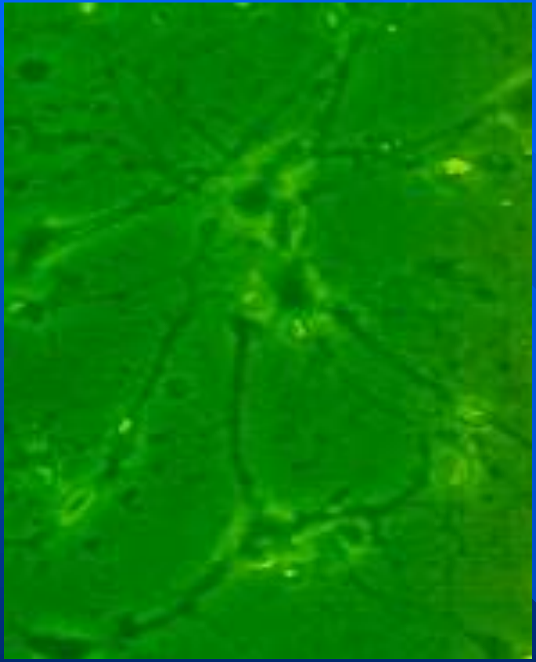


培養 7 天

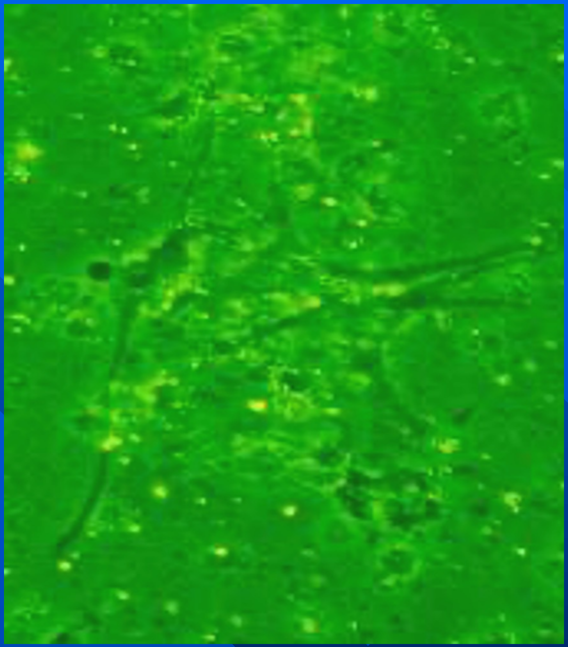


培養 14 天

10 μM Aβ1-42
誘發海馬迴神經細胞凋亡



Normal

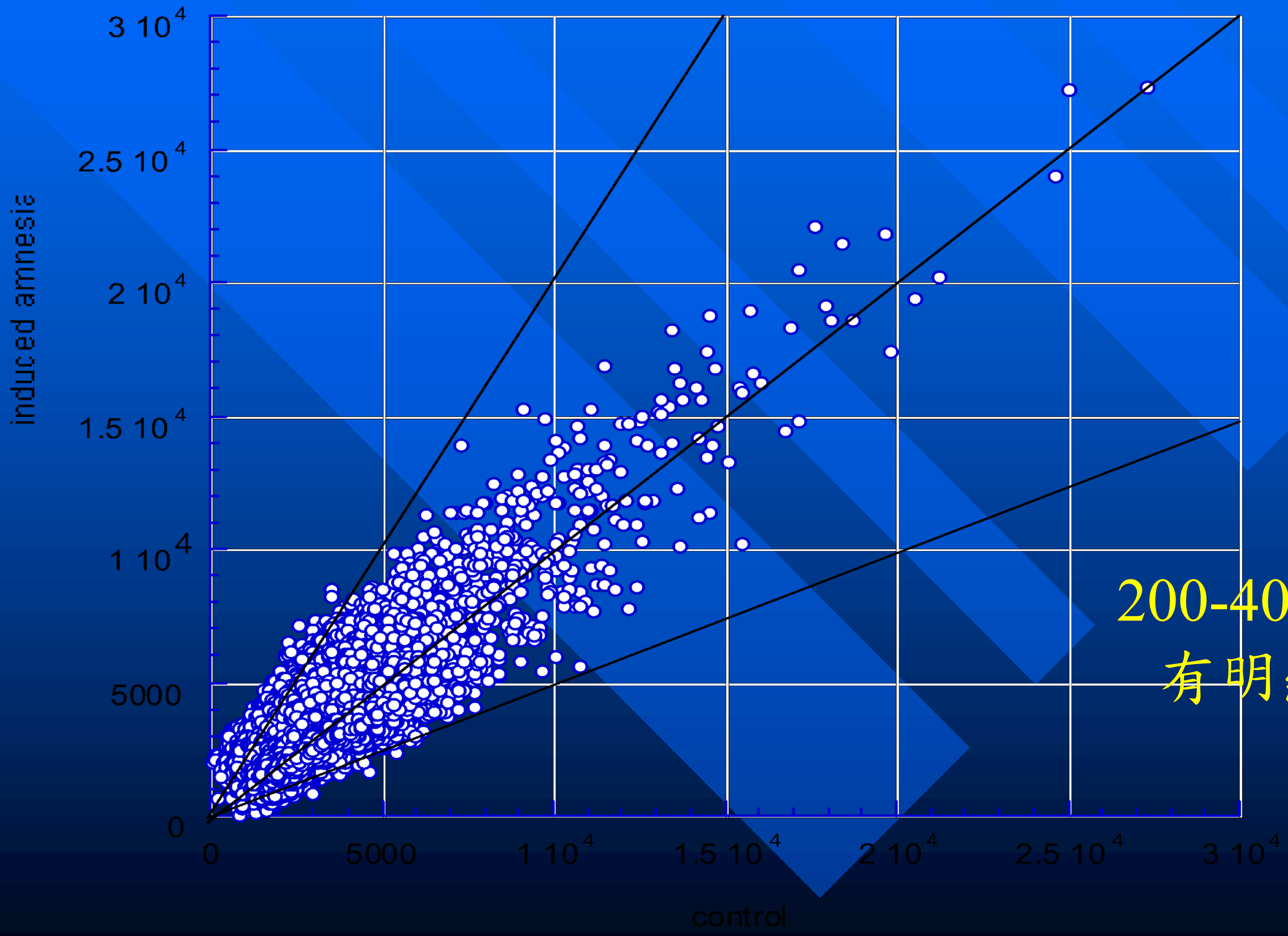


10 μM Aβ1-42

結　論

- 地黃成分catalpol
  - 增強正常大鼠於水迷宮學習反應之能力
  - 改善因 scopolamine 誘發健忘大鼠水迷宮學習障礙之作用
  - 改善因類澱粉樣蛋白誘發痴呆大鼠水迷宮學習障礙之作用
  - 改善因腦缺血誘發痴呆大鼠水迷宮學習障礙之作用
    - » 與增加 hippocampus 、cortex 區 norepinephrine 濃度有關
    - » 與減少 cortex 區 dopamine 濃度有關

SCOPOLAMINE VERSUS CONTROL IN CORTEX



200-400個基因
有明顯變化

表現增加基因

R-5.11-2	Title
2.3105	FK506-binding protein 4 (59kD)
2.0629	adhalin (DAG2)
2.0447	Follicular lymphoma variant translocation 1
1.9744	EST
1.9639	ESTs
1.9374	Selectin P ligand
1.9134	PKU-alpha
1.8305	KIAA0463 protein
1.8294	EST
1.8059	ESTs
1.78	"ubiquitous TPR motif, X isoform (UTX), alt"
1.7759	KIAA0079 gene
1.7745	Hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndro
1.7596	KIAA0185 gene
1.7588	clone 24761 sequence
1.7478	insulinoma rig-analog encoding DNA-binding protei
1.7414	12p13.3 BAC RPCI11-283I3 (Roswell Park Cancer Institute BAC Library)
1.737	"ESTs, Highly similar to TUBULIN BETA CHAIN [Schizosaccharom"
1.7185	KDEL endoplasmic reticulum protein retention receptor 3 (KDELR3)
1.7146	V-yes-1 Yamaguchi sarcoma viral related oncogene homolog
1.7052	MAJOR HISTOCOMPATIBILITY COMPLEX ENHANCER-BINDING PROTEIN MA
1.6988	SP1-like zinc finger transcription factor (TIEG
1.6893	Integrin alpha-3 subunit
1.6842	"ESTs, Weakly similar to PROTEIN A55 [Vaccinia virus (strain "
1.6748	Protein S (alpha)
1.6594	adenine nucleotide translocase
1.6542	Carboxypeptidase A1
1.6483	"ESTs, Highly similar to ELONGATION FACTOR G, MITOCHONDRIAL "
1.6461	ESTs
1.646	mitochondrion genome
1.6407	"Collagen, type VI, alpha 2"
1.6392	"ESTs, Highly similar to PROCOLLAGEN ALPHA 2(IV) CHAIN PRECU"
1.6353	Aldehyde dehydrogenase 7
1.6345	X104
1.6335	Synaptophysin
1.6292	insulin-like growth factor II
1.6274	clone 23614 sequence
1.6273	KIAA0014 gene
1.6257	"Protein phosphatase 1, catalytic subunit, beta isoform"
1.621	(clone pA3) protein disulfide isomerase related protei
1.6171	MATRIN 3
1.6168	HEAT SHOCK PROTEIN HSP 90-ALPHA
1.6129	ESTs
1.6104	high density lipoprotein binding protein (HBP)
1.6023	2'-5' oligoadenylate synthetase 59 kDa
1.6004	"Actin depolymerizing factor [human, fetal brain,, 1452 "PRECU"
1.5926	KIAA0287 gene
1.5912	STATHMIN
1.5904	"ESTs, Highly similar to MICROSOMAL SIGNAL PEPTIDASE 23 KD S"
1.5892	"Homo sapiens chromosome 18, clone RP11-349N23"
1.5892	no find*
1.5885	KIAA0695 protein
1.5867	"Adaptin, beta 1 (beta prime)"
1.5862	U1 SMALL NUCLEAR RIBONUCLEOPROTEIN A
1.5828	Activated leucocyte cell adhesion molecule
1.5792	vanin-like genes
1.5779	"ESTs, Highly similar to GLUTATHIONE PEROXIDASE [Schistosoma"
1.5776	Met proto-oncogene (hepatocyte growth factor receptor)
1.5754	"Wingless-type MMTV integration site 5A, homolog"
1.5745	"Vitronectin (serum spreading factor, somatomedin B"
1.5719	ESTs
1.5624	ESTs
1.5614	KIAA0095 gene
1.5603	clone 23649 and 23755 unknown
1.5601	Apolipoprotein H
1.5592	no find*
1.5561	"ESTs, Highly similar to myc-associated zinc-finger protein"
1.5458	"Calponin 3, acidic"
1.5455	"Solute carrier family 9 (sodium/hydrogen exchanger), isoform"
1.5418	ATRIAL NATRIURETIC FACTOR PRECURSOR
1.541	succinyl CoA:3-oxoacid CoA transferase precursor (OXCT
1.5397	Annexin II (lipocortin II)
1.5388	Thrombomodulin
1.5386	Int-6
1.5375	DR-nm23
1.537	"ESTs, Weakly similar to PHOSPHATIDYLETHANOLAMINE-BINDING PRO"
1.5351	Orosomucoid 1
1.5349	HKL1
1.5343	Homo sapiens 12p13 BAC RPCI11-233G1 (Roswell Park Cancer Institute Human BAC Library)
1.5313	chromosome X map Xp11.23 containing VDAC and SALL1P pseudogenes sequence
1.53	KIAA0974 protein
1.5271	latent transforming growth factor beta binding protein 1 (LTBP1)
1.5259	"ESTs, Highly similar to TRANSCRIPTIONAL ACTIVATOR GCN5 [Sac"
1.525	"EST, Weakly similar to hypothetical protein"
1.5234	"ESTs, Weakly similar to MATRIN 3"
1.5218	ESTs
1.5212	Feline sarcoma (Snyder-Theilen) viral (v-fes)/Fujinami avian
1.5211	ESTs
1.5197	CDC46 HOMOLOG
1.519	V-rel avian reticuloendotheliosis viral oncogene homolog
1.5183	VRK1

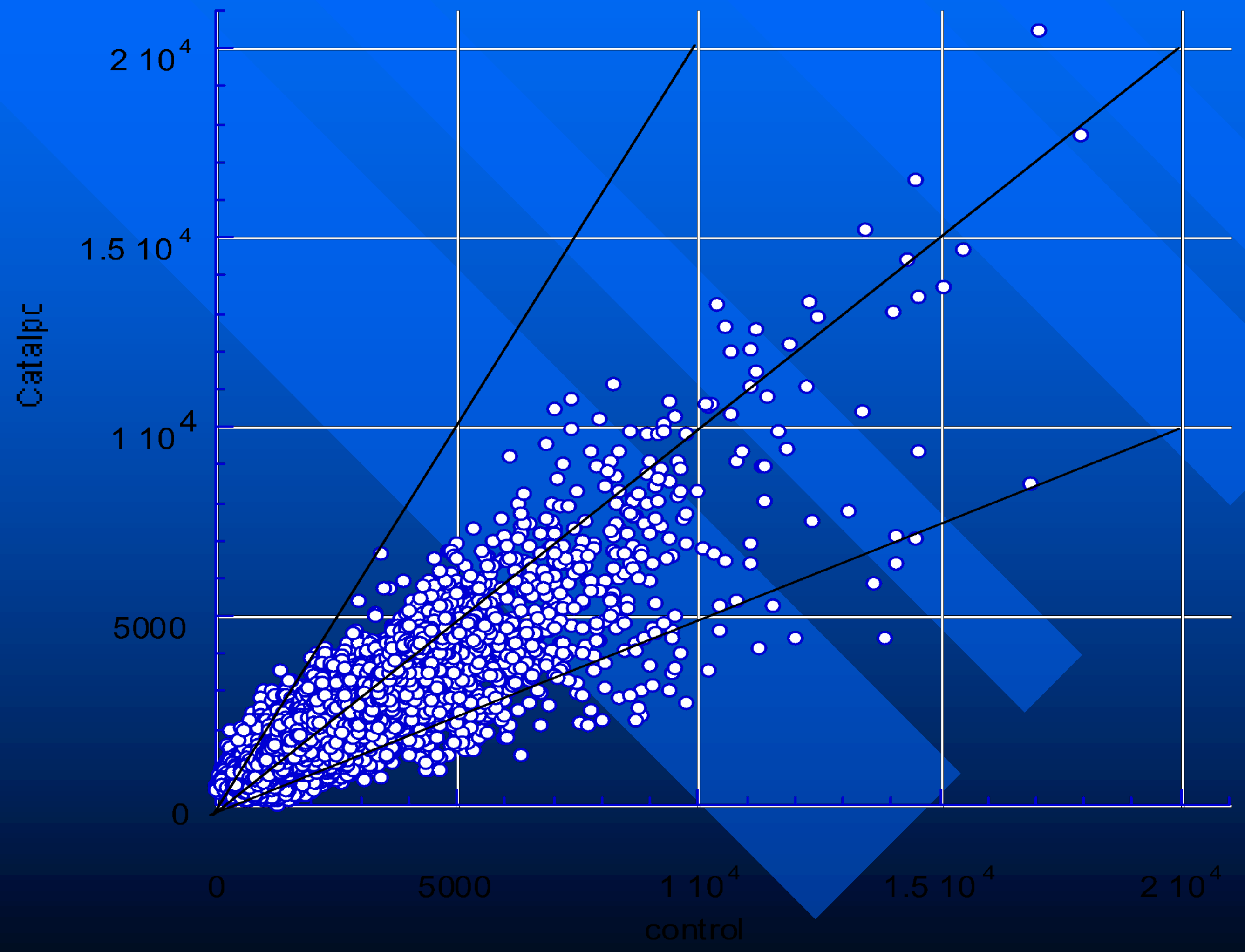
0.55104	Catechol-O-methyltransferase
0.5506	NADH:ubiquinone oxidoreductase B17 subunit
0.5503	Heat shock 60 kD protein 1 (chaperonin)
0.54931	N-ACETYLLACTOSAMINE SYNTHASE
0.54822	no find*
0.54802	Rattus norvegicus vacuolar ATPase subunit F (vatf)
0.54669	DNA recombination and repair protein (MRE11B) m
0.54666	P55-C-FOS PROTO-ONCOGENE PROTEIN
0.54661	Fc-gamma RIII-2 cDNA for Fc-gamma receptor III-2 (CD16)
0.54619	no find*
0.54599	acyl Co-A: cholesterol acyltransferase-2 (ACAT2
0.54578	Cut (Drosophila)-like 1 (CCAAT displacement protein)
0.54517	ESTs
0.5435	nitrilase 1 (NIT1)
0.54308	ESTs
0.54215	Patched (Drosophila) homolog
0.54138	"Pyruvate kinase, muscle"
0.54078	perilipin
0.53711	SERYL-TRNA SYNTHETASE
0.53533	semaphorin (CD100)
0.53403	Adenomatosis polyposis coli
0.53368	EST
0.53368	Prostaglandin E receptor 3 (subtype EP3) {alternative produc
0.5325	EST
0.53116	ESTs
0.52879	ADP-ribosyltransferase (NAD+; poly (ADP-ribose) polymerase)
0.52817	"mRNA, clone:PO2ST9"
0.52684	"ESTs, Highly similar to NUCLEAR MOVEMENT PROTEIN [Emericell"
0.52606	skeletal muscle 190kD protein
0.52581	"ESTs, Highly similar to ACETYL-COENZYME A SYNTHETASE"
0.52184	ESTs
0.52127	Apolipoprotein H
0.51912	"ESTs, Highly similar to ZINC FINGER PROTEIN ZFP-35 [Mus mus"
0.51882	"ESTs, Weakly similar to HYPOTHETICAL 16.1 KD PROTEIN IN SEC1"
0.51852	G-rich RNA sequence binding factor 1
0.5176	"Uncoupling protein 2 (mitochondrial, proton carrier)"
0.51552	Zinc finger protein 42 (myeloid-specific retinoic acid-respo
0.51515	Polypyrimidine tract binding protein (hnRNP I)
0.51458	Keratin 13
0.51142	"potassium large conductance calcium-activated channel, subfamily M, beta1 (KCNMB1)"
0.50996	Thrombopoietin (myeloproliferative leukemia virus oncogene l
0.50944	"EST, Moderately similar to LINE-1 REVERSE TRANSCRIPTASE HOM"
0.50886	"ESTs, Highly similar to PROTEIN PHOSPHATASE PP2A, 72 KD REG"
0.50872	clone 23967 unknown
0.44724	V-myb avian myeloblastosis viral oncogene homolog-like 1
0.44643	"ESTs, similar to HEMOGLOBIN EPSILON-4 CHAIN"
0.44605	nicotinamide N-methyltransferase (NNMT)
0.44466	DNA-binding protein (SMBP2)
0.44419	795G23 Contains T-Box (Brachyury) family
0.44361	COP9 homolog (COP9)
0.44165	"Casein kinase 2, beta polypeptide"
0.43928	Adenylosuccinate lyase
0.43634	"PEPTIDYL-PROLYL CIS-TRANS ISOMERASE, MITOCHONDRIAL PRECURSOR"
0.43252	"Actin, alpha 2, smooth muscle, aorta"
0.42481	IMP (inosine monophosphate) dehydrogenase 2
0.41667	Nuclear factor of kappa light polypeptide gene enhancer in B
0.41648	"ATPase, Na+/K+ transporting, alpha 1 polypeptide"
0.4147	autoantigen p542
0.40033	KIAA0260 gene
0.39084	MXI1
0.39057	APOLIPOPROTEIN AI REGULATORY PROTEIN-1
0.38992	ESTs
0.37588	KIAA0268 gene
0.36616	Esterase D/formylglutathione hydrolase
0.36006	ribosomal protein L23-related
0.33925	"ESTs, Highly similar to NEUROLYSIN PRECURSOR [Sus scrofa]"
0.30615	"matrix associated, actin dependentregulator of chromatin, subfamily c, member 1 (SMARCC1)"
0.30002	H.sapiens Pur (pur-alpha)
0.26401	ESTs

表現減低基因

Special genes in SCOP-treated rats

- coagulation factor C homolog (Limulus polyphemus)
- phosphoglycerate mutase 1
- Burkitt lymphoma receptor 1
- glypican 3
- epidermal growth factor receptor
- coronin, actin binding protein 1B
- secretory carrier membrane protein 2
- deiodinase, iodothyronine, type II

CATALPOL VERSUS CONTROL IN CORTEX



總計畫經費動支暨學校補助情形

	第一年			第二年		
	國科會補助經費	學校配合款	計畫執行支用數	國科會補助經費	學校配合款	計畫執行支用數
研究人力費	1,600,000		1,600,000	1,640,000		1,640,000
研究設備費	1,100,000	650,000	1,100,000	1,000,000	1,000,000	1,000,000
其他研究有關費用	1,800,000	800,000	1,800,000	1,760,000	540,000	1,760,000
管理費	270,000		270,000	352,000		352,000
合計	4,770,000	1,450,000	4,770,000	4,752,000	1,540,000	4,752,000

具體成果

- 專利申請：共 1 件
  - 用於增強智能之藥學組合物
  - 申請案號：91105124
- 論文發表：共 14 篇
  - 已接受 11 篇、投稿中 3 篇
- 研討會發表：共 10 篇

第三年度預定執行項目

- 以細胞培養探討地黃成分 catalpol 改善記憶作用之分子機制
- 探討地黃成分 catalpol 對大鼠海馬迴乙醯膽鹼釋放之影響
- 以 microarray 及 RT-PCR 探討地黃成分 catalpol 改善記憶作用與基因表達之關係

謝 謝